CLINICAL BIOCHEMISTRY

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PREFACE TO THE SEVENTH EDITION

In producing the seventh edition of this book I am greatly indebted to my colleagues in the Departments of Biochemistry and Physiology.

To Professor V. M. Trikojus, in whose Biochemical Department much endocrine research is in progress, I owe my thanks for having rewritten portion of the chapter on endocrine glands and also for his expert advice concerning blood analysis. Professor R. D. Wright has largely rewritten the chapter on water and inorganic electrolytes.

Associate Professor W. A. Rawlinson, Mr J. W. Legge and Mr Ian Parsons have made most valuable contributions to the discussion of haemoglobin and its related pigments and to blood and blood pigments in urine. Dr C. W. Crane, who has made a special study of hepatic disorders, has reconstructed the chapter on liver disease and liver function tests and has added much new material of great interest. Dr J. Bornstein has given his special knowledge in bringing up to date the sections on glycosuria, pancreatic efficiency and acidosis and Dr Vera Krieger of the Royal Women's Hospital has made many suggestions which are incorporated in the discussion of renal efficiency.

I should like to thank Miss M. Bailie and Dr Murray Maxwell for reading the proofs and Miss Bailie also for describing the photoelectric colorimeter and for her work in preparing the index. Others to whom I am indebted for criticism of the text are Miss Beryl Splatt, Dr Alan Mackay and Mr J. Somerset.

Finally, it is a pleasure to express my appreciation of the interest in the book shown by my publishers, the Melbourne University Press.

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THE INVESTIGATION OF GASTRIC FUNCTION BY TEST MEALS

INTRODUCTION

Clinical biochemistry has become an essential part of the investigation of many pathological conditions. By its use the diagnosis, prognosis and treatment of disease has been immeasurably improved. The information obtained by clinical biochemistry must be carefully appraised. It is often only one link in a chain of evidence which the clinician assembles and studies before arriving at a diagnosis.

FUNCTIONS OF THE STOMACH

(A) PEPTIC DIGESTION

The glands of the fundus and body of the stomach contain three types of cells:

(a) parietal or oxyntic cells which are concerned with the formation of hydrochloric acid;
(b) chief cells which secrete the proteoclastic enzyme pepsin;
(c) mucous cells found at the neck of the gland tubules and on the surface epithelium of the gastric mucosa and which are engaged in the formation of a mucous secretion.

It is most important to realize that the glands of the pyloric region do not form either hydrochloric acid or pepsin, but secrete an alkaline fluid rich in mucin. In man there is evidence that the body of the stomach and to a less extent the duodenum are the sites of formation of the intrinsic factor of Castle. The glands in the immediate vicinity of the cardiac orifice are of the mucous type and form an alkaline fluid. The secretion of hydrochloric acid and that of pepsin are independent of one another. Hydrochloric acid may be markedly diminished in some circumstances, whilst the pepsin remains normal in amount. Stimulation of the vagus nerves normally causes secretion of a juice rich in pepsin

and strongly acid (0·4 to 0·5 per cent HCl). This is referred to as psychic or appetite juice.

The chemical transmitter of the vagus to the stomach is acetylcholine. The vagi probably control the secretion of mucus by the surface epithelium of the gastric mucosa and the mucous "neck cells". The sympathetic fibres are regarded by most observers as being inhibitory to the peptic and oxyntic cells, but cause secretion of an alkaline mucoid juice from the pyloric glands. A further factor involved in gastric secretion is a hormone, named gastrin by Edkins which is formed chiefly in the pyloric region and to a less extent in the duodenum and carried in the circulation to the body and fundus of the stomach, stimulating the cells in these areas – particularly the oxyntic cells – to activity (see Fig. 1). Gastrin is a protein of low molecular weight. It is free from histamine and closely resembles secretin in its chemical properties.

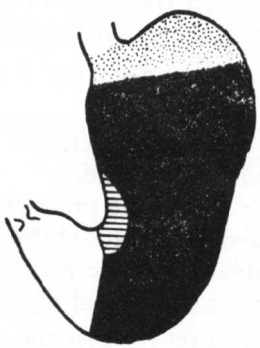

FIG. 1. Diagram showing the distribution of the parietal (acid-secreting) cells in the human stomach. In the black area the proportion of parietal cells was maximal and was taken as 100 per cent; in the shaded area on lesser curvature the percentage of parietal cells was 75 per cent, in the dotted area at the fundus 50 per cent, and in the white area 0 to 1 per cent. (After Berger. (1934), *Amer. J. Anat. 54*, 87).

Another factor which influences gastric secretion is the percentage of sugar in the blood. A fall in blood sugar, e.g. following the injection of insulin, stimulates the vagal nucleus and induces

increased gastric secretion of both HCl and pepsin. Gastric secretion is inhibited by a rise in blood sugar.

Fat inhibits gastric secretion, particularly the intestinal phase. It reduces the quantity and acidity of the juice and depresses especially its peptic power. According to Ivy a hormone which has been named enterogastrone is produced in the intestinal mucosa as a result of the action of fat. This hormone when conveyed in the blood to the stomach may cause suppression of gastric secretion lasting from one to four hours and markedly inhibits gastric motility. A hormone which may be named "enterogastrin" is probably produced in the duodenum and plays a prominent part in the stimulation of gastric secretion during the intestinal phase of stomach activity.

Hydrochloric acid acts as an activator of pepsin and the latter converts protein into acid meta-protein, proteose and peptone. If pepsin is rendered inactive owing to the absence of hydrochloric acid, digestion of protein occurs in the small intestine under the influence of trypsin which acts in an alkaline or neutral medium and may complete the hydrolysis to the stage of polypeptides and amino acids.

(B) SECRETION OF RENNIN

This is a milk-curdling enzyme and is thought to be formed by the chief cells of the body of the stomach. It converts calcium caseinogenate into calcium caseate, which is insoluble and is responsible for the physical characters of the curd.

(C) SECRETION OF GASTRIC LIPASE

This is a weak fat splitting enzyme differing from that secreted by the pancreas in that it acts in an acid medium, the optimum pH being 4 to 5. It ceases to act at pH 2·5. It is probably not of great importance in gastric digestion, but is stated to be of some value in the hydrolysis of highly emulsified fat, such as is found in milk and yolk of egg.

(D) SECRETION OF THE "INTRINSIC FACTOR"

According to Castle "pernicious anaemia would not develop if the patient could effect daily the transfer of a millionth of a gram of vitamin B_{12} the distance of a small fraction of a milli-

metre across the intestinal mucosa and into the blood stream". By use of the gastroscope or by gastric biopsy (Wood *et al.*) or at post-mortem examination it is found that the region of the gastric mucosa which is atrophied in pernicious anaemia is that in which the glands normally secrete HCl and pepsin.

Achlorhydria is present in patients suffering from pernicious anaemia and it is common in the relatives of persons suffering from this disease. Complete achylia frequently occurs. It has been shown by Castle *et al.* that 150 ml. to 300 ml. of normal human gastric juice secreted under the stimulus of histamine and then introduced into the stomach of a patient suffering from pernicious anaemia was without effect, as was also 200 grams of beef muscle even after complete digestion with pig pepsin. But when beef muscle and normal gastric juice were given together they produced a marked haemopoietic effect. Apparently there is an interaction between something in normal gastric juice (intrinsic factor) and a constituent (extrinsic factor) in muscle which is responsible for normal haemopoiesis. The extrinsic factor is now known to be B_{12} (cyanocobalamin). It is produced by certain bacteria found in the alimentary tract of man and animals and is stored in such foods as meat (muscle), liver and other cellular organs. Gastric juice freed from pepsin and rennin still contained the intrinsic factor. Boiling the gastric juice destroys the intrinsic factor. It is now thought to be a muco-polysaccharide. The intrinsic factor in man is secreted chiefly in the main body of the stomach but perhaps to some extent in the duodenum.

Various theories have been suggested as to the mode of action of the intrinsic factor on the extrinsic factor. The decreased faecal excretion of radio-active cobalt that results from the oral administration of intrinsic factor together with cyanocobalamin (B_{12}) suggests that the intrinsic factor facilitates the absorption of the extrinsic factor.

The loss of intrinsic factor in the gastric secretion is a slow and progressive process and the lack of this factor appears to be a quantitative rather than a qualitative effect. The amount of gastric secretion in pernicious anaemia averages about 20 ml. per hour compared with 150 to 160 ml. per hour in a normal person.

Clinical experience in America and Britain indicates th crystalline B_{12} given intra-muscularly every three or four we

in a dose averaging 1 to 2μg. (1 to 2 microgrammes) daily is adequate therapy. Vitamin B_{12} seems preferable to liver extract in pernicious anaemia because it causes less discomfort at the site of injection, does not give rise to untoward reactions and is less expensive.

Hyperchromic macrocytic anaemia may be due to one or more of the following causes:

(a) Deficiency in the secretion of the "intrinsic" factor. This occurs in pernicious anaemia.

(b) Deficiency of "extrinsic" factor in the food. This is exhibited in poorly fed people and is common in India. It may be cured by the administration of Vitamin B_{12} or by the use of "Marmite" or "Vegemite" which contain the "extrinsic" factor.

(c) Deficient absorption of B_{12} from the alimentary tract manifested in chronic diarrhoea, coeliac disease and sprue.

(d) Failure of the liver to store B_{12} which sometimes occurs in cirrhosis of the liver accompanied by gross hepatic inefficiency.

It is obvious from this summary that hyperchromic anaemia may have a diverse clinical syndrome and the diagnosis can only be accurately determined by careful examination of the blood and of the patient.

(E) ANTISEPTIC ACTION

Hydrochloric acid in normal concentration in the stomach destroys many micro-organisms including streptococci which are swallowed with saliva from the mouth and with mucus from the naso-pharynx and tonsils. Diseased tonsils and inflammatory conditions of the maxillary antra and ethmoid sinuses greatly add to the number of bacteria entering the stomach. There is evidence that various infections of the bowels, such as dysentery and typhoid fever, occur more frequently in persons with achlorhydria than in normal individuals. The duodenum is normally comparatively sterile, but in achlorhydria bacillus coli may invade this region from the colon. Chronic diarrhoea is frequently associated with achlorhydria.

TEST MEALS

The following procedures are in use:

the one-hour gruel test meal;
the fractional gruel test meal;
the alcohol test meal;
the histamine test;
the insulin test;
the combined insulin and histamine test;
tubeless gastric analysis.

The One-hour Test Meal

In this method the patient who has fasted since the previous evening is given at 9 a.m. or some other convenient time a meal consisting of a pint of gruel (for preparation see p. 9). The contents of the stomach are aspirated exactly one hour later and analysed. It should be noted that no aspiration is performed before giving the gruel meal.

The one-hour test meal is open to various objections, the chief of which are:

1. A single analysis at the end of one hour does not give a clear indication of what is happening in the stomach during the preceding 60 minutes, nor does it reveal what may occur during the subsequent period till the natural emptying of the stomach is completed.

2. No information is obtained as to the volume or composition of the fasting gastric contents.

3. Gastric analysis gives figures which are used mainly for comparative purposes, and hence the stomach should be, as far as possible, under identical conditions before the test meal is given. Since the resting gastric contents varies markedly in volume and composition in different individuals, this variable factor should be eliminated by removing the resting juice before the test meal is given, otherwise this residue when mixed with the ingested meal may modify considerably the composition of the material subsequently aspirated.

4. The rate of emptying of the stomach cannot be accurately determined in the one-hour method.

For these reasons most clinicians prefer the fractional method.

The Fractional Test Meal

In this method an attempt is made to follow the various phases of gastric digestion. A rubber tube of small bore is passed into the stomach and any residuum present removed. The tube is left in position and the meal swallowed. Every 15 minutes after taking the meal a sample of gastric contents is removed, until the stomach is empty; analyses of the samples are then made.

Ryle has laid emphasis on the following advantages of the fractional method:

1. It enables an accurate study to be made of the fasting gastric contents and allows pathological constituents in this or in subsequent aspirated specimens to be detected.

2. It allows the details of gastric secretion to be followed during the whole stage of digestion, and late rises in acidity and prolonged secretion to be detected.

3. The time at which biliary regurgitation occurs can be noted.

4. The emptying rate of the stomach is determined.

The chief criticisms of the fractional method are:

1. The tube acts as a foreign body in the stomach and may induce abnormal secretion. It has, however, been demonstrated that inert foreign bodies in the stomach do not influence its secretory activity to any marked degree.

2. The tube stimulates excessive salivation, and the saliva, if swallowed, would dilute and partially neutralize the gastric juice. To avoid this, patients are provided with a vessel into which saliva is expectorated.

3. Psychical inhibition of the gastric secretion may occur as a result of anxiety or fear. This does not seem to be a serious criticism, except in a very occasional patient.

4. It is stated that variations occur from day to day in the gastric curve of an individual. This may be so, but the variations are usually of no great magnitude. It has been observed that, in patients on whom the test meal was performed daily for several days, the curve was lower on the first occasion than on subsequent days. If a low curve is obtained in a patient with symptoms suggestive of hyperchlorhydria, it is well to repeat the test next day.

5. It is urged that the gastric contents at any time are not